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SOMATOSTATIN ANALOGS FOR DIAGNOSIS AND TREATMENT OF CANCER

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Abstract—Somatostatin (SRIF) is a cyclic tetradecapeptide hormone initially isolated from ovine hypothalami. It inhibits endocrine and exocrine secretion, as well as tumor cell growth, by binding to specific cell surface receptors. Its potent inhibitory activity, however, is limited by its rapid enzymatic degradation and the consequent short plasma half-life. Octreotide is a short SRIF analog with increased duration of action compared to SRIF. Octreotide is approved for the treatment of acromegaly, amine precursor uptake and decarboxylation-omas, complications of pancreatic surgery and severe forms of diarrhea. Preclinical studies have focussed on the anticancer effects of octreotide and the related SRIF analogs BIM 23014 and RC-160. *In vitro* at nanomolar concentrations, these analogs inhibit the growth of tumor cells that express high affinity SRIF receptors. Accordingly, SRIF analogs, such as octreotide, potently inhibit the growth of SRIF receptor-positive tumors in various rodent models, and, in particular, xenotransplanted human tumors in nude mice. The range of cancers susceptible to octreotide and related SRIF analogs includes mammary, pancreatic, colorectal and lung malignancies. Moreover, an indirect antiproliferative effect of SRIF analogs is achievable in SRIF receptor-negative tumors, whose growth is driven by factors (gastrin, insulin-like growth factor-1, etc.) that are downregulated by SRIF. The use of radiolabeled somatostatin analogs represents a new diagnostic approach. [^{111}In-DTPA]octreotide was developed for gamma camera imaging of SRIF receptor-positive malignancies, such as gasteroenteropancreatic tumors. Visualization of SRIF receptor-positive tumors in humans is emerging as an important methodology, both in tumor staging and predicting therapeutic response to octreotide. Recently, five SRIF receptor subtypes (SSTR1-5) have been cloned, all of which bind SRIF with high affinity. In contrast, SRIF receptor subtypes 1-5 have different binding profiles for short SRIF analogs. Octreotide, RC-160 and BIM 23014 are very similar in that they bind with high affinity to SSTR2 and SSTR5, show moderate affinity for SSTR3 and fail to bind with high affinity to the other subtypes (SSTR1 and 4). Accordingly, the oncological profile of these three analogs is apparently similar. In conclusion, somatostatin analogs are a promising class of compounds for diagnosis and treatment of cancer. Current work is focussed on the identification of further SRIF receptor subtype-selective analogs with potential in oncology.

Keywords—Somatostatin receptor subtypes, octreotide, oncology/cancer, receptor imaging/scintigraphy, cell culture, animal tumor models.

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Abbreviations—DMBA, dimethylbenzanthracene; DTPA, diethylenetriaminepentaacetic acid; GH, growth hormone; IGF-1, insulin-like growth factor-1; IGFBP, IGF-binding protein; PTPase, phosphotyrosine phosphatase; SCLC, small cell lung carcinomas; SRIF, somatotropin-release inhibiting factor; SSTR1–5, somatostatin receptor subtypes 1–5.

1. INTRODUCTION

Somatostatin (somatotropin-release inhibiting factor, SRIF) is a cyclic peptide hormone that is widely distributed throughout the central nervous system and the gastrointestinal system (Reichlin, 1983; Patel, 1987; Gyr and Meier, 1992). In mammals, there are two forms of somatostatin (SRIF-14 and SRIF-28) that are produced by tissue-specific proteolytic cleavage of a common prohormone precursor (preprosomatostatin) (Danoff *et al.*, 1991). SRIF is known to inhibit the secretion of multiple hormones (e.g. growth hormone (GH), insulin, glucagon, gastrin), gastric acid and pancreatic enzymes (Tannenbaum *et al.*, 1990; Thorner *et al.*, 1990). In the central nervous system, SRIF can act as a neurotransmitter and may affect locomotor activity and cognitive functions (Bell and Reisine, 1993). Somatostatins bind to at least five different subtypes of specific, high-affinity SRIF receptors located on the target cells. The recent cloning of these five SRIF receptor subtypes (SSTR1–5), is opening up new avenues for diagnosis and therapy of various diseases (Bell and Reisine, 1993; Bruns *et al.*, 1993a).

The effects of natural somatostatins are limited by the rapid proteolytic degradation in the blood (plasma half-life ~ 1 min). Consequently, the therapeutic application of SRIF requires continuous infusion regimens. Therefore, a number of SRIF analogs with increased plasma half-life have been synthesized. Three particular cyclooctapeptide analogs, namely octreotide (SMS 201-995, Sandostatin®), somatuline (BIM 23014) and vapreotide (RC-160), have been investigated in depth (Bauer *et al.*, 1982; Marbach *et al.*, 1985; Schally, 1988; Parmar *et al.*, 1989; Evers *et al.*, 1991; Pless, 1992). The structures of these three analogs are shown in Fig. 1. Octreotide exhibits a markedly increased stability in the circulation (plasma half-life ~ 2 hr) (Marbach *et al.*, 1992). Consequently, it is an important therapeutic agent (O'Dorisio, 1989) and has been approved for the treatment of acromegaly, tumors of the amine precursor uptake and decarboxylation system (glucagonomas, carcinoid, etc.), complications of pancreatic surgery and severe forms of diarrhea. RC-160 and BIM 23014 are currently being studied in various phases of clinical trials.

Various findings have led to a general interest in the possible oncological utility of SRIF analogs: (i) the discovery of an antiproliferative action of SRIF-14 on HeLa and gerbil fibroma tumor cells *in vitro* (Mascardo and Sherline, 1982), (ii) the shrinkage of pituitary and carcinoid tumors in response to octreotide treatment and (iii) the detection of various somatostatin receptor-positive human tumors, such as breast and lung malignancies (Lamberts *et al.*, 1991). Tumor-associated SRIF receptors are not only important for mediating growth inhibition, but they can also serve as targets for SRIF receptor scintigraphy, a novel diagnostic approach for tumor localization in cancer patients (Lamberts *et al.*, 1991).

Over the last three years, important information has become available concerning (i) the range of experimental cancers susceptible to octreotide, BIM 23014 and RC-160, (ii) the underlying mode of the anticancer action of somatostatin analogs, (iii) the use of labeled SRIF analogs for the diagnosis of SRIF receptor-positive tumors and their metastases and (iv) the relevance of SRIF receptor subtypes. Our review will address the recent progress made in these fields.

TABLE 1. *Effect of SRIF Analogs on Tumor Cell Growth* In Vitro

Cancer	SRIF receptor status	Analog	Growth inhibition	Reference
BREAST CANCER				
Estrogen receptor positive cells				
MCF-7 human	K_d 0.07 nM B_{max}	SMS	Conc.-dep.	Setyono-Han *et al.*, 1987
	57 kDa with crosslinking	BIM	Conc.-dep.	Prévost *et al.*, 1992
ZR-75-1 human	K_0.9 nM B_{max} 6,000 r/c	SMS, RC	Conc.-dep. at 0.01–100 nM	Weckbecker *et al.*, 1992b
Estrogen receptor negative cells				
MDA-231 human	not detectable	SMS	none	Weckbecker *et al.*, 1992b
	57 kDa with cross linking	BIM	none	Prévost *et al.*, 1992
MDA-436 human	not done	SMS	≈35% at 500 nM	Nelson *et al.*, 1989
GASTRIC CANCER				
MKN45G human	K_d 1.4 nM B_{max} 330,000 r/c	SMS	conc. dep. at 0.2–200 nM	Watson *et al.*, 1992
COLORECTAL CANCER				
LIM 1215 human	not done	SMS	conc. dep. at 0.002–200 nM	Dy*et al.*, 1992
DHD/K12 rat	not done	RC	conc. dep. at 10–1000 nM	Qin *et al.*, 1992
PANCREATIC CANCER				
AR42J rat	IC_{50} 0.2 nM B_{max} 1.1 pmol/mg	SMS	conc. dep. at 0.001–1000 nM	Viguerie *et al.*, 1989
		RC	0.1–1000 nM	Gillespie *et al.*, 1992
MIA PaCa-2 human	K_d 4.3 nM B_{max} 1.75 pmolmg	RC	0.001–1 nM	Radulovic *et al.*, 1993; Liebow *et al.*, 1989
LUNG CANCER				
NCI-H69	positive	BIM	at 10 nM	Taylor *et al.*, 1988
HX149 human	positive	SMS	conc. dep. 0.1 and 1 nM	Macaulay *et al.*, 1991
PROSTATIC CANCER				
LNCap	not done	RC	at 0.1 nM	Gattani *et al.*, 1990
CERVICAL CANCER				
HeLa human	not done	SRIF	conc. dep. 0.1 fM-10 nM	Mascardo and Sherline, 1982

Abbreviations: RC, RC-160; SMS, SMS 201-995, octreotide; BIM, BIM 2310; R/C, receptors/cell.

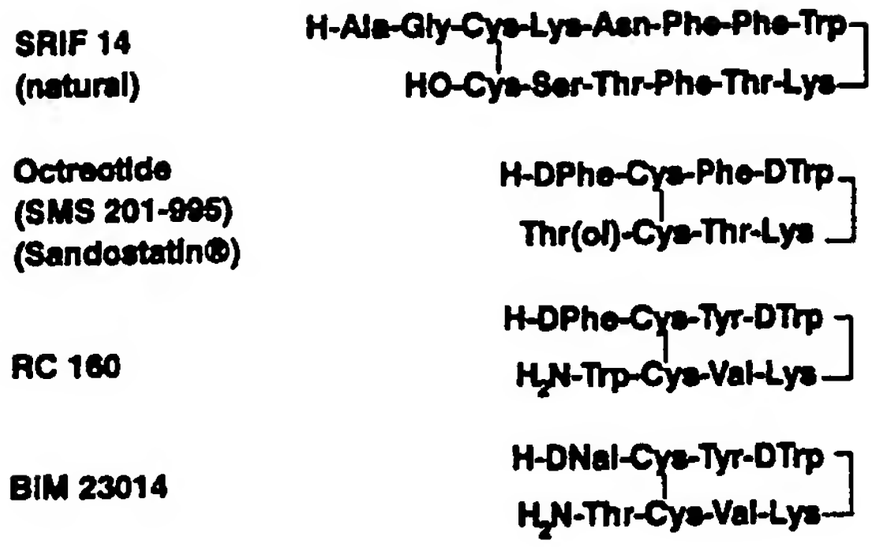

FIG. 1. Structures of somatostatin 14, octreotide, RC-160 and BIM 23014.

2. ACTIVITY OF SOMATOSTATIN ANALOGS IN VARIOUS CANCER MODELS

2.1. IN VITRO ACTIVITY OF SOMATOSTATIN ANALOGS

The somatostatin analogs octreotide, BIM 23014 and RC-160 have been extensively tested *in vitro* with regard to their antiproliferative properties (Table 1). Either animal or human cancer cell lines were used in proliferation assays. The extent of cell growth inhibition was determined by measuring parameters, such as cell count, cell protein or thymidine incorporation, after exposure of the test cell lines to nanomolar concentrations of the analogs. A number of cell lines inhibited by SRIF analogs were also shown to be SRIF receptor-positive. Thus, the antiproliferative action of the SRIF analogs is obviously directly mediated by high-affinity SRIF receptors on these tumor cells.

The relevance of SRIF receptors for the antiproliferative action of SRIF analogs is further supported by *in vitro* studies using 7315b pituitary tumor cells (Hofland *et al.*, 1992). The number of high-affinity SRIF receptors in these cells increases with the length of the *in vitro* culture period; immediately after harvesting of cells from rats, SRIF receptors were not detectable, but they steadily increased within the following 3 weeks of culture to levels of 5.9 pmol/mg protein. Importantly, there was a significant positive correlation between the extent of cell growth inhibition and SRIF receptor density in 7315b cells. Similarly, SRIF receptor-negative cell lines, such as the human mammary cancer cell line MDA-MB-231, failed to be growth inhibited by even high concentrations (1 μM) of octreotide (Weckbecker *et al.*, 1992b).

Controversial results were obtained in various laboratories with MIA PaCa-2 cells *in vitro*. For example, Gillespie *et al.* (1992) could detect neither SRIF receptors nor an effect of RC-160 on the growth of MIA PaCa-2 cells. By contrast, Radulovic *et al.* (1993) found the same cell line both to be growth inhibited by RC-160 and also to express binding sites for radiolabeled RC-160 (K_d 4.3 nM, 75,000 sites/cell or 1.75 pmol/mg protein). Such differences, observed for SRIF receptor expression and cell growth behavior in the presence of somatostatin analogs, might be due to fluctuations in SRIF receptor density or subtype expression, which could be a function of cell handling, source of media and sera, passage number, etc. The currently increasing understanding of SRIF receptor expression and regulation will certainly contribute to optimization of experimental conditions for analyzing the antiproliferative effects of SRIF analogs.

Because octreotide, BIM 23014 and RC-160 are structurally related (cf. Fig. 1), the range of their biological activities, as well as their potency, is comparable, if not identical, in a number of endocrine and oncological models (Parmar *et al.*, 1989; Klijn *et al.*, 1990; Woltering *et al.*, 1991; Weckbecker *et al.*, 1992b,c). This view was further supported when we compared side-by-side the effects of BIM 23014, RC-160 and octreotide on the growth of various tumor cell lines. For example, in AR42J rat pancreatic tumor cells, which express high levels of somatostatin receptors (Viguerie *et al.*, 1989), the extent of cell growth inhibition was similar for the three analogs (Fig. 2).

In conclusion, comprehensive *in vitro* studies have established a role of SRIF and selected analogs as potent inhibitors of the growth of SRIF receptor-positive tumor cells. Cancer cell lines

responding *in vitro* to SRIF analogs include lines derived from mammary, gastric, colorectal, pancreatic, lung, prostatic and cervical tumors.

2.2. In Vivo Activity of Somatostatin Analogs

The somatostatin analogs octreotide, BIM 23014 and RC-160 have been studied in various rodent cancer models, with special emphasis on human tumor xenotransplants in nude mice. The effect of these analogs was determined by recording the change in tumor volume during and after treatment. Only a few studies addressed the inhibitory effect of SRIF analogs on carcinogenesis or metastatic spread. The therapy with SRIF analogs was often started when tumors were rather small. The SRIF analogs were administered either by daily to twice daily bolus injection or by continuous infusion using osmotic minipumps. The doses reported to be effective in the various models vary significantly, but generally there is a tendency to use high-dose regimens such a 10 μg/kg/hr (infusion) or 2.5 mg/kg (bolus injection).

We determined the plasma levels of octreotide during continuous infusion under conditions that led to significant inhibition of tumor growth (Weckbecker *et al.*, 1992b). An infusion rate of 10 μg/kg/hr octreotide can maintain plasma levels of 5.7 ng/mL. For comparison, octreotide plasma levels as low as 0.2–0.4 ng/mL are sufficient to induce a half-maximal decrease in plasma GH levels in rats, dogs and rhesus monkeys (Marbach *et al.*, 1992).

Frequently, the outcome of somatostatin treatment has been related to the SRIF receptor status of the tumors being treated. Opposed to the *in vitro* situation, where only SRIF receptor-positive tumor cells are sensitive to SRIF analogs, tumors with and without specific, high affinity SRIF receptors can both respond *in vivo* to treatment with SRIF analogs. Accordingly, the *in vivo* antitumor effects of SRIF analogs can be either direct, in SRIF receptor expressing tumors, or indirect, when tumors lack SRIF receptors.

The following paragraphs provide an overview of direct and indirect tumor growth-inhibitory effects of octreotide, BIM 23014 and RC 160 *in vivo*. Please note: If the SRIF receptor status of the tumor used in the various studies is not mentioned below, it has not yet been investigated.

2.2.1. *Mammary Cancer*

Breast cancer is the most common malignancy in women. The interest in studying the effects of SRIF analogs in breast cancer models *in vivo* is due to (i) the detection of a high incidence of SRIF receptor-positive mammary tumors (Reubi *et al.*, 1990b) and (ii) early studies showing that the growth of human breast cancer cells *in vitro* is inhibited by SRIF analogs (Setyono-Han *et al.*, 1987).

The growth of the human estrogen-dependent breast tumor MCF-7 in nude mice was

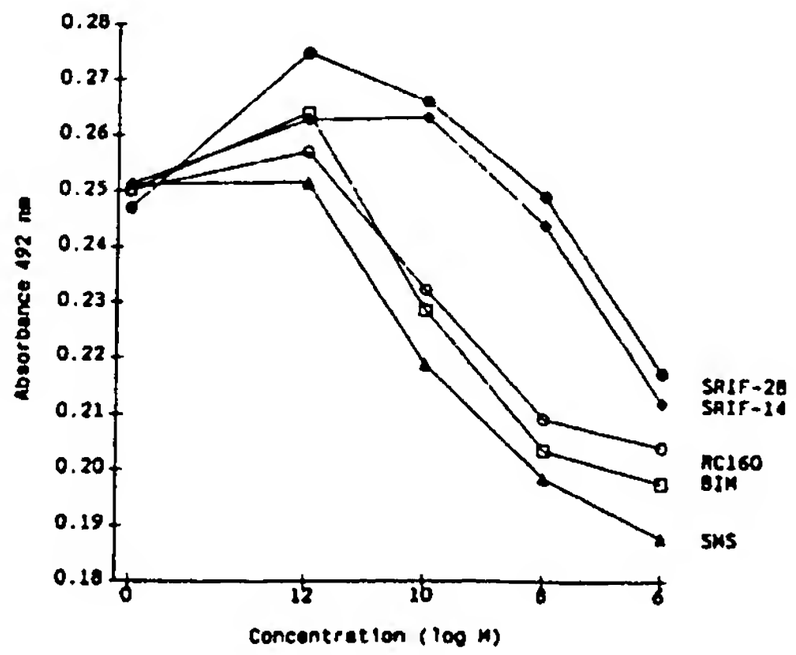

FIG. 2. Effects of octreotide (SMS), RC-160, BIM 23014, (BIM), SRIF-14 and SRIF-28 on the growth of AR-42J rat pancreatic tumor cells. Cell growth was determined in 96-well plates using the sulforhodamine B (SRB) assay.

significantly retarded by twice daily injection of octreotide (200 μg/kg), with tumor doubling time being increased from 13.2 days (control) to 19 days (octreotide) (Weber *et al.*, 1989). Similarly, BIM-23014 reduced the growth of MCF-7 tumors in a nude mouse subrenal capsule assay (Prévost *et al.*, 1992). In the latter study a ligand cross-linking procedure was used to show that the tumors expressed binding sites for SRIF-14 and BIM 23014.

We used ZR-75-1 human breast tumors (estrogen-dependent), since ZR-75-1 cells are growth inhibited by octreotide *in vitro* and express high affinity binding sites for octreotide (cf. Table 1) (Weckbecker *et al.*, 1992b). Continuous infusion of octreotide at 10 μg/kg/hr in nude mice bearing ZR-75-1 tumors induced a highly significant inhibition of tumor growth over a 4-week treatment period. Of the ZR-75-1 tumors studied *ex vivo*, about 70% were SRIF receptor positive, as determined by SRIF receptor autoradiography.

Indirect effects may also play an important role in treatment of mammary cancer with SRIF analogs. Dimethylbenzanthracene (DMBA)-induced rat tumors are heterogenous in SRIF receptor expression, but they responded to continuous treatment with octreotide (10 μg/kg/hr) in that the tumor multiplicity (number of tumors per animal) was reduced (Weckbecker *et al.*, 1992c). This indicates that octreotide may be more active in the initial phase of tumorigenesis. In line with our data, Liebow *et al.* (1993) showed that RC-160 apparently reverses the development of malignancies initiated by local administration of DMBA. Conflicting results (inhibition and lack of inhibition) were obtained in the DMBA model with bolus injection of octreotide (Setyono-Han *et al.*, 1987; Bakker *et al.*, 1990b).

MDA-MB-468 human breast tumors growing in nude mice is another example of the indirect effects of octreotide. Using receptor autoradiography, we recently demonstrated that MDA-MB-468 lacked specific high-affinity SRIF receptors, but the tumor growth was significantly inhibited by either continuous administration or bolus injection of octreotide (Weckbecker *et al.*, 1992a).

Mammary cancer frequently progresses from an estrogen-dependent more benign form to an estrogen-independent aggressive form. From the limited data obtained in models of estrogen-dependent mammary cancer (ZR-75-1, MCF-7, DMBA) and estrogen-independent cancer (MDA-MB-468), it appears that somatostatin analogs, such as octreotide, could be useful for therapy of both types of breast cancer.

2.2.2. *Colorectal Cancer*

Colorectal cancer represents a largely unmet medical need where standard cytotoxic agents, such a 5-fluorouracil alone or in combination with other drugs, lack beneficial activity in the majority of patients and surgery is still the first line of therapy. Currently, SRIF analogs are being studied as potential drugs for treatment of colon cancer.

Smith and Solomon (1988) showed that twice daily injections of 100 and 300 μg/kg SRIF-14, dissolved in hydrolyzed gelatine to prolong absorption, induced significant inhibition of the growth of human colon adenocarcinomas (CX1) in nude mice. Dy *et al.* (1992) found that continuous administration of octreotide at daily doses of 5–50 μg/kg caused a significant dose-related inhibition of the growth of human colorectal tumor xenotransplants (LIM 2405 and LIM 2412). Long-term treatment with RC-160 (50 μg/kg b.i.d.) inhibited the growth of liver metastases induced by intrasplenic injection of DHD/K12 colon adenocarcinoma cells into syngeneic BDIX rats (Qin *et al.*, 1992). Since DHD/K12 cells were also growth inhibited *in vitro*, a direct SRIF receptor-mediated effect can be assumed.

An indirect inhibitory effect of octreotide on tumor growth was apparently obtained with murine colonic adenocarcinoma cells CT-26 (Alonso *et al.*, 1992). Cell proliferation *in vitro* was not inhibited by octreotide, but the growth of CT-26 tumors in mice was potently suppressed, indicating an inhibitory effect of octreotide on growth factors and hormones stimulating CT-26 tumor growth *in vivo*.

These results are encouraging; clinical trials will show whether SRIF analogs, such as octreotide, are useful for treating patients with colorectal cancer (Dy *et al.*, 1992). Since indirect antiproliferative effects of SRIF analogs may play an important role, failure of colorectal tumors to express SRIF receptors should not automatically be regarded as an exclusion criterion for SRIF analog based therapies.

2.2.3. *Pancreatic Cancer*

The very poor prognosis of pancreatic cancer (5-year survival rate is less than 1%) reflects the inadequacy of current therapies. Studies in different animal models have addressed the potential role of SRIF analogs in the treatment of pancreatic cancer. Octreotide inhibited the growth of two xenotransplanted human pancreatic adenocarcinomas (Upp *et al.*, 1988). Hajri *et al.* (1991) showed that octreotide (infused at a rate of 40 μg/kg/day) could significantly retard the growth of rat pancreatic acinar tumors over a 15-day treatment period. Using receptor autoradiography, SRIF receptors were detected on this tumor. Importantly, octreotide treatment did not downregulate the SRIF receptors of the tumors; in control and octreotide-treated tumors, the K_d for SRIF was 0.16 and 0.17 nM and B_{max} 100 and 110 fmol/mg protein, respectively.

Szepeshazi *et al.* (1991) characterized RC-160 as an inhibitor of nitrosamine-induced pancreatic cancers in hamsters. Tumor incidence and tumorous pancreas weight were potently decreased by high-dose RC-160 (1.5 mg/kg/day). Histological evaluation showed an increase in the number of apoptotic tumor cells under RC-160 treatment.

The growth of MIA PaCa-2 tumors (derived from a human pancreatic cancer cell line) was dose-dependently inhibited in nude mice by twice daily injections of octreotide (250 and 2500 μg/kg) (Weckbecker *et al.*, 1992a). Correspondingly, microcapsules of RC-160 releasing 25 μg/day (1250 μg/kg) significantly inhibited tumor growth in nude mice bearing MIA PaCa-2 tumors (Radulovic *et al.*, 1993).

2.2.4. *Small Cell Lung Carcinoma*

Despite the clinical relevance of small cell lung carcinomas (SCLC) and the unambiguous demonstration of SRIF receptor expression by SCLC in patients (Lamberts *et al.*, 1991), effects of SRIF analogs on lung cancer has not received much attention at the preclinical level. Bogden *et al.* (1990) treated four human SCLCs growing in athymic nude mice with BIM 23014. This SRIF analog was administered b.i.d. as an ultra high dose (25 mg/kg) either on the side opposite the tumor or as an infusion around the tumor. All tumors responded. In particular, the perilesional administration of BIM 23014 led to potent tumor growth inhibition. Tumors, such as NCI-H345 SCLC, responded exclusively to the perilesional regimen, which could indicate that only high concentrations of the analog at the tumor site can exert significant tumor growth-inhibitory effects. This cell line was SRIF receptor-positive, suggesting direct effects of BIM 23014 on tumor growth. However, other SCLC cell lines (LX-1 and NCI-N417), which responded *in vivo*, expressed only very low levels of SRIF receptors; accordingly, indirect effects may play an important role in the antitumor action of SRIF analogs (Bogden *et al.*, 1990).

2.2.5. *Prostate Cancer*

Cancer of the prostate is the most common cancer in American men, with a 5-year survival between 30 and 60%. It has been suggested that somatostatin analogs may be useful for therapy of prostate cancer (Schally, 1988). However, only a small number of studies support this concept. The SRIF octapeptide analog DC-13-135 [H-(D)Cpa–Cys–Tyr–(D)Trp–Lys–Val–Cys–Thr(NH_2)], which was given at a daily dose of 100 μg/kg, reduced the weight of R-3327 rat prostate tumors (Dunning) by 41% (Murphy *et al.*, 1987). Using the same tumor model, Siegel *et al.* (1988) evaluated octreotide effects. Treatment was started when tumors were already rather large (700 mm^3) and tumor growth was followed by means of magnetic resonance imaging. After a 3-week treatment period with octreotide (100 μg/kg b.i.d.), tumor growth was inhibited by 42%. Interestingly, an interruption of treatment led to regrowth of the tumors, but when treatment was resumed, tumor growth was again inhibited.

2.2.6. *Early Treatment*

The initiation of cancer therapy with SRIF analogs is optimal when tumors express high levels of SRIF receptors. However, tumors change their properties during progression. For example, prostate and breast cancers express receptors for androgens and estrogen, respectively, in an early

stage, but they frequently become hormone-insensitive in an advanced stage of the disease because of the loss of the respective hormone receptors. Similarly, SRIF receptors seem to be useful markers of a less malignant, more differentiated stage of a tumor (Lamberts *et al.*, 1991). Moreover, advanced tumors are heterogenous, i.e. they consist of different cell types that may vary quantitatively and even qualitatively in their range of enzymes, receptors or antigens. All this information strongly argues for an early onset of octreotide therapy when tumors are still small, containing homogeneously expressed SRIF receptors and tumor spread is absent or still at the stage of micrometastases. An advanced tumor that is heterogenous in the expression of SRIF receptors may only partly be responsive to SRIF analogs.

3. POSSIBLE MECHANISMS INVOLVED IN THE ANTICANCER EFFECTS OF SOMATOSTATIN ANALOGS

SRIF analogs may inhibit the growth of SRIF receptor-positive tumors by triggering signal transduction pathways that negatively control cell growth (*direct mechanism*). In addition, they may inhibit the proliferation of SRIF receptor-negative tumors by downregulating stimuli of tumor growth, such as hormones and growth factors (*indirect mechanism*). The concept of the direct and indirect actions of SRIF analogs offers a comprehensive explanation for the anticancer activity of SRIF analogs. However, many aspects need to be further confirmed.

3.1. DIRECT EFFECTS

SRIF receptor-mediated triggering of signalling cascades that lead to inhibition of cell division appears to require a minimal amount of high affinity SRIF receptors per target cell. This is indicated by a study in prolactin secreting 7315b rat pituitary cells (Hofland *et al.*, 1992). The number of SRIF receptors on these cells increases with the length of the *in vitro* culture period. The inhibition of 7315b cell growth by octreotide required higher numbers of SRIF receptors than the inhibition of prolactin secretion.

The SRIF receptor-mediated control of tumor cell growth regulation includes the activation of phosphotyrosine phosphatases (PTPases) and the inhibition of adenylyl cyclase.

3.1.1. *Phosphotyrosine Phosphatases*

Phosphorylation of tyrosine residues on proteins involved in the signal transduction pathways is essential in the regulation of cellular proliferation and differentiation (Hunter, 1987, 1989). Tyrosine kinases and PTPases catalyze the phosphorylation and dephosphorylation reactions, respectively. In general, activation of tyrosine kinases can be involved in the stimulation of cell growth and their overexpression or permanent activation can lead to cellular transformation. By contrast, PTPases can abrogate cell growth (Pot and Dixon, 1992).

Reyl and Lewin (1982) were the first to report SRIF receptor-mediated activation of a PTPase. The enzyme they studied is a cytoplasmic PTPase occurring in normal pancreas. Liebow *et al.* (1989) showed that RC-160 and SRIF-14 both stimulated a PTPase and exerted antiproliferative effects in MIA PaCa-2 cells. A 70-kDa plasma membrane PTPase was characterized in AR42J rat pancreatic tumor cells, which are growth inhibited by octreotide (Tahiri-Jouti *et al.*, 1992). This PTPase could be stimulated in a time- and dose-related manner in intact AR42J cells by octreotide, maximal activation being obtained at 0.1 nM. These data were further supported by investigations in rat pancreatic acini (Colas *et al.*, 1992). In this system, too, exposure to octreotide or BIM 23014 led to an increase in membrane PTPase activity, with maximal activation occurring at 0.1 and 0.1–1.0 nM, respectively. Thus, PTPases may play a role in the antimitogenic signalling pathways triggered via SRIF receptors. Since AR42J cells overexpress one SRIF receptor subtype, SSTR2, it may be concluded that growth inhibition in this system requires the formation of a complex consisting of ligand/SSTR2/G-protein resulting in activation of a PTPase as the target effector

system. In a preliminary study, Srikant* hints that octreotide may modulate the cdc25 tyrosine phosphatase, an enzyme that is necessary for progression through the cell cycle. In addition, octreotide interfered in a cell cycle-dependent manner with EGF receptor autophosphorylation. Activation of tyrosine kinases was also reported by Srikant (1993). The intricate network of phosphorylation and dephosphorylation reactions affected by SRIF analogs requires further study to elucidate the role of phosphoproteins in the regulation of tumor cell growth by SRIF analogs.

3.1.2. *cAMP*

SRIF has long been known to affect cellular contents of cAMP. The second messenger cAMP has a dual effect on cell growth in that it can inhibit proliferation (Tortora *et al.*, 1991) or stimulate it (Dumont *et al.*, 1989). Since cAMP can exert a negative control on cell proliferation, cAMP analogs, such as 8-chloro-cAMP, are currently being tested as anticancer agents in preclinical and clinical studies (Tortora *et al.*, 1991). In contrast, in certain mammalian cell lines, cAMP stimulates proliferation and can act as a mediator of mitogenic hormones. For example, the functional and growth-promoting action of thyrotropin is mediated by cAMP (Dumont *et al.*, 1989). A significant percentage of pituitary tumors (ca. 40%) in acromegalics have markedly elevated cAMP levels, which suggests that increased contents of cAMP do not prevent the growth of these tumors (Landis *et al.*, 1989). It is unclear, at the moment, whether the pituitary tumors with elevated cAMP are the ones that are growth-inhibited by long-term treatment with octreotide. Since SRIF analogs always lower cAMP levels, they may inhibit the growth of those cells that respond to proliferative actions of cAMP. However, in rat pancreatic tumor cells (AR42J), changes in cAMP levels are not involved in octreotide-induced inhibition of cell proliferation (Viguerie *et al.*, 1989). While this does not prove that cAMP is generally irrelevant to the antiproliferative action, it shows that other mechanisms may be important as well. The importance of cAMP for SRIF receptor-mediated inhibition of cell growth is still unclear.

3.2. Indirect Effects

The indirect effect of somatostatin analogs may involve suppression of growth factors and hormones that stimulate tumor growth. Insulin-like growth factor-1 (IGF-1) is important in this context, since it potently stimulates the growth of various types of cancer, e.g. breast and lung cancer (Macaulay, 1992). Moreover, the IGF-1 levels in blood and peripheral tissues are controlled by SRIF in a GH-dependent and possibly in a GH-independent fashion.

The GH-dependent mechanism involves expression of IGF-1 in hepatocytes stimulated by GH. Since GH secretion is inhibited by SRIF, SRIF analogs also diminish the hepatic production of IGF-1 (Thorner *et al.*, 1990; Tannenbaum *et al.*, 1990). In acromegalics, octreotide inhibits the excessive GH secretion by the pituitary tumor and leads in parallel to reduced IGF-1 blood levels. For example, in the 25 patients studied by Lamberts *et al.* (1988), mean IGF-1 levels dropped from 6.9 U/mL before to 2.7 U/mL during treatment with octreotide. Ongoing clinical trials with octreotide in breast cancer patients have similarly demonstrated a lowering of IGF-1 plasma levels (Pollak *et al.*, 1992).

A GH-independent control of IGF-1 expression by SRIF and SRIF analogs was suggested by Serri *et al.* (1992), who studied the effects of octreotide and exogenous GH on IGF-1 expression in hypophysectomized rats. When these animals received human GH, the expression of hepatic IGF-1 increased 4-fold, while the combination of human GH and octreotide was not associated with increased hepatic IGF-1 levels. This GH-independent inhibitory effect of octreotide on IGF-1 expression may also be relevant for the antitumor activity of octreotide.

Since IGF-1 action is modulated by its association with IGF-binding proteins (IGFBPs), the effects of octreotide on IGFBP-1 expression has also been examined. Ren *et al.* (1992) showed that nanomolar concentrations of octreotide increased basal and cholera toxin-stimulated IGFBP-1 levels up to 4-fold in human hepatic tumor cells (Hep G2). This finding may be relevant for the anticancer action of octreotide since IGFBP-1 can inhibit the growth of IGF-dependent cancer cells *in vitro*.

*Srikant, C. B. (1993) Somatostatin inhibition of tumor cell growth and induction of apoptosis: cell cycle-specific regulation of distinct phosphotyrosine proteins. 75th Annual Meeting of The Endocrine Society, Las Vegas, NV, June 9–12, 1993, Abstract 478.

Another indirect effect of SRIF analogs may involve the inhibition of tumor-associated angiogenesis. Since the growth of tumors heavily depends on the efficient supply of essential nutrients and growth factors via the blood, inhibitors of angiogenesis have been proposed as promising novel drugs with relatively high tumor selectivity (Folkman, 1990). Woltering *et al.* (1991) compared RC-160 and octreotide as potential inhibitors of vessel growth in the chorioallantoic membrane assay. The analogs were adsorbed to methylcellulose disks and implanted on the chorioallantoic membrane of chick embryos. Following exposure to either octreotide or RC-160 (0.5, 2.5, 50 μg/disk), the overall incidence of inhibition was determined to be 13, 56, 61, 27, 49 and 68%, respectively. Since these data point to an additional, rather important facetto of the anticancer action of SRIF analogs, further studies are warranted to establish the relevance of these findings for tumor therapy.

4. TUMOR IMAGING WITH RADIOLABELED SOMATOSTATIN ANALOGS

Many human tumors and their metastases express specific high affinity receptors for somatostatin (SRIF). This fact was established by *in vitro* receptor-binding studies with gasteroenteropancreatic tumors, breast cancer, some brain tumors and SCLC (Lamberts *et al.*, 1990a; Reubi *et al.*, 1990a). An exciting area in nuclear medicine is the development of a new class of radiopharmaceuticals based on somatostatin analogs.

Receptor scintigraphy with gamma-emitter labeled SRIF analogs is a very sensitive method for imaging of SRIF receptor-positive tumors and their metastases in patients (Lamberts *et al.*, 1990a; Bakker *et al.*, 1991b; Krenning *et al.*, 1992b). The presence of high affinity SRIF receptors on the tumor cell is a prerequisite for the visualization of the tumor tissue *in vivo* using radiolabeled SRIF analogs. The first successful visualization of primary SRIF receptor-positive tumors, as well as their metastases, was achieved with an iodinated SRIF analog, [^{123}I-Tyr3]-octreotide (Lamberts *et al.*, 1990a; Krenning *et al.*, 1992a). The Tyr3-octreotide analog, when labeled with the gamma-emitting isotope ^{123}I, has the same biological activity and similar affinity for the SRIF receptor when compared with somatostatin (Bakker *et al.*, 1990a). The specificity of this new *in vivo* imaging technique was demonstrated by the parallel *in vitro* detection of SRIF receptors on tumor tissue samples by autoradiography (Lamberts *et al.*, 1990a).

Although successful tumor imaging results were obtained with [^{123}I-Tyr3]-octreotide, various factors, such as (i) the relatively short effective half-life of this compound, (ii) the high background of radioactivity in the abdomen due to its hepato-biliary clearance and (iii) the metabolic cleavage of the iodinated octreotide, are drawbacks to its application (Bakker *et al.*, 1991b). Furthermore, its preparation is cumbersome and requires some radiochemical expertise. To overcome these considerable drawbacks, a diethylenetriaminepentaacetic acid (DTPA)-coupled somatostatin analog (DTPA-octreotide, SDZ 215-811) was developed.

DTPA has already been used to chelate metallic cations, such as the gamma-emitter ^{111}In, to antibodies (Fairweather *et al.*, 1983). Furthermore, the ^{111}In-DTPA antibody conjugates were shown to be suitable for kit formulations designed for in-house labeling (Bunn *et al.*, 1984).

The SRIF receptor selectivity, the high binding affinity and the SRIF-like biological behavior of DTPA-octreotide indicate that DTPA-octreotide retains SRIF-like activity (Bakker *et al.*, 1991a; Bruns *et al.*, 1993b). ^{111}In is incorporated into the DTPA moiety of SDZ 215-811 with greater than 95% efficiency in an easy, single step labeling procedure that requires further purification. [^{111}In]SDZ 215-811 ([^{111}In]DTPA-octroetide, OctreoScan®^{111}) showed a slightly lower affinity when compared with the iodinated Tyr3octreotide, but it still binds with nanomolar affinity (Bakker *et al.*, 1991a).

In vivo, [^{111}In]DTPA-octreotide can be used to visualize SRIF receptor-positive tumors efficiently 24 hr after injection, when interfering background radioactivity has been minimized by renal clearance of the radioligand. This renal clearance has a significant advantage over the previously used [^{123}I-Tyr3]-octreotide, which causes a high abdominal interference due to its hepato-biliary clearance.

Successful imaging requires target to background ratios of at least 2:1; ratios of 5:1 are necessary to detect deeper and smaller lesions (Rockoff *et al.*, 1980). Favorable ratios were achieved for

TABLE 2. *Incidence of SRIF Receptor Positive Tumors*

Tumor	[^{111}In-DTPA]octreotide detection rate (%)	Reference
Paragangliomas	87–100	Krenning *et al.*, 1992c; Kwekkeboom *et al.*, 1993
Glioma grade III, IV	100	Schmeidhauer *et al.*, 1993
Malignant lymphoma	75–100	Bares *et al.*, 1993
Medullary thyroid ca.	70	Dörr *et al.*, 1993b
Gastrinoma	88–100	Krenning et al., 1992c; Joseph *et al.*, 1993
Insulinoma	57	Krenning *et al.*, 1992
Carcinoid	87–100	Krenning *et al.*, 1992c; Joseph et al., 1993
Functionally non-active endocrine tumors	92	Joseph *et al.*, 1993
Small cell luing cancer	100	Krenning *et al.*, 1992c
Breast carcinoma	78	Krenning *et al.*, 1992c

[^{111}In-DTPA]-octreotide in the animal models studied. Tumor to blood ratios of 1.6:1 (1 hr post injection) and 4.9:1 (24 hr post injection) (Bruns *et al.*, 1993b) demonstrated that [^{111}In-DTPA]-octreotide is a valuable tool for *in vivo* imaging of even small SRIF receptor-positive tumors and their metastases. The *in vivo* specificity of this new radiopharmaceutical was proven in tumor bearing rats pretreated with 1 mg/kg octreotide. The accumulation of [^{111}In-DTPA]-octreotide in the SRIF receptor-positive tumors was blocked in the pretreated animals, indicating receptor specificity of the radiopharmaceutical *in vivo* (Bakker *et al.*, 1991c; Bruns *et al.*, 1993b).

Compared with labeled antibodies, the plasma clearance of the labeled hormone analogs is fast (Bakker *et al.*, 1991c; Krenning *et al.*, 1992a). [^{111}In-DTPA]-octreotide was rapidly cleared from the circulation during the first few minutes and excreted via the kidneys (Dörr *et al.*, 1993a). Within 24 hr after injection, about 68% of the applied dose was excreted in the urine (Adrian *et al.*, 1993). In contrast, the more lipophilic [^{123}I-Tyr3]-octreotide is metabolized via the liver. [^{111}In-DTPA]-octreotide undergoes glomerular filtration and tubular back-resorption (Bakker *et al.*, 1991c), which explains the high retention and residence time of activity in the kidney. The estimated absorbed doses for [^{111}In-DTPA]-octreotide in non-target tissues were 0.07 mGy/MBq (liver), 0.32 mGy/MBq (spleen) and 0.45 mGy/MBq (kidney) in patients with SRIF receptor-negative tumors (Krenning *et al.*, 1992a). However, patients with large neuroendocrine tumors positive for SRIF receptors showed a lower radiation dose in the non-target organs (Adrian *et al.*, 1993). The calculated effective radiation dose equivalent for tumor-free patients was 0.08 mGy/MBq (Krenning *et al.*, 1992a; Adrian *et al.*, 1993; Joseph *et al.*, 1993).

Detection rates up to 100% have been reported for a variety of human tumors (Table 2). In the majority of these tumors, a good correlation was found between the results of the *in vivo* scintigraphy and the parallel *in vitro* somatostatin receptor autoradiography with the respective biopsies (Lamberts *et al.*, 1990b; Krenning *et al.*, 1992c; Reubi *et al.*, 1992). In patients with amine precursor uptake and decarboxylation-cell-derived tumors, the detection of SRIF receptors with [^{111}In-DTPA]octreotide seems to predict the possibility of octreotide therapy to control symptoms caused by hypersecretion of hormones from these tumors (Lamberts *et al.*, 1990b). However, a prospective investigation of the inhibitory effect of octreotide on the growth of metastatic endocrine gasteroenteropancreatic tumors demonstrated that the presence of octreotide receptors does not predict an antiproliferative effect of octreotide therapy (Arnold *et al.*, 1993).

The concept of radiodiagnosis with radiolabeled SRIF analogs has recently been expanded to a gallium chelating analog [Desferrioxamine B-succinyl-(D)Phe1]-octreotide (SDZ 216-927)* (Smith-Jones *et al.*, 1993; Mäcke *et al.*, 1993). This analog can be labeled with the gamma-emitting isotope 67Ga for conventional imaging or with 68Ga for positron emission tomography. Positron

*Smith-Jones, P., Stolz, B., Bruns, Ch., Albert, T., Reist, H. W., Fridrich, R. and Mäcke, H. (1994) 67/68Ga(Desferrioxamine B-succinyl-(D)Phe1]-octreotide a potential radiopharmaceutical for PET imaging of somatostatin receptor-positive tumors: synthesis, radiolabeling, *in vitro* and preliminary *in vivo* studies. *J. Nucl. Med.*, accepted.

emission tomography with [68Ga]SDZ 216-927, for the first time, enables quantitative receptor imaging of SRIF receptors *in vivo*.

Considering the rapidly expanding research on SRIF receptor subtypes, it is conceivable that future development of receptor subtype-selective SRIF analogs for tumor imaging could result in more specific diagnostic radioligands for human tumors expressing different SRIF receptor subtypes (Bell and Reisine, 1993; Bruns *et al.*, 1993a).

As already evaluated for use with antibodies or antibody fragments (e.g. Buchegger *et al.*, 1986), a radiotherapeutic isotope might be coupled to octreotide-chelate conjugates. Due to their relatively large molecular mass (104–105 kDa) the plasma clearance of antibodies or antibody fragments is slow. SRIF analogs that have a significantly lower molecular mass (103 kDa) are rapidly cleared from the circulation with a half-life time of some minutes. Consequently, radiotherapeutic treatment with radiolabeled antibodies leads to a higher total body irradiation than treatment with radiolabeled hormone analogs, such as octreotide derivatives. Hence, an opportunity for local and specific therapeutic irradiation of SRIF receptor-positive tumors can be foreseen. This approach would be especially interesting for SRIF receptor-positive tumors that do not respond to Sandostatin® treatment (Arnold *et al.*, 1993) and/or inoperable cancers with advanced metastases.

5. MOLECULAR BIOLOGY OF THE SOMATOSTATIN RECEPTOR GENE FAMILY

5.1. Molecular Cloning of Five SRIF Receptor Subtypes

An expanding gene family of SSTRs has recently been cloned. These distinct SRIF receptors may mediate the various biological effects of the peptide hormone and neurotransmitter somatostatin. The different cloned SRIF receptor subtypes were numbered according to the order in which they were reported. The first receptor identified, hSSTR1 (Yamada *et al.*, 1992a), was cloned by reverse transcriptase-polymerase chain reaction with primers corresponding to highly conserved amino acid sequences in the third and sixth transmembrane region of the members of the superfamily of G-protein coupled seven-helix membrane-spanning receptors (GPRs). Other subtypes were successively cloned by screening genomic libraries with SSTR1 probes (Yamada *et al.*, 1992a; Rohrer *et al.*, 1993), expression library cloning (Kluxen *et al.*, 1992) or further polymerase chain reaction approaches (Meyerhof *et al.*, 1992).

At the moment there are five known subtypes:

In humans: hSSTR1, hSSTR2 (Yamada *et al.*, 1992a), hSSTR3 (Yamada *et al.*, 1992b) and hSSTR4 (Rohrer *et al.*, 1993).

In mice: mSSTR1 and mSSTR2 (Yamada *et al.*, 1992a) and mSSTR3 (Yasuda *et al.*, 1992).

In rat: rSSTR1 (Li *et al.*, 1992), rSSTR2 (Kluxen *et al.*, 1992), rSSTR3 (Meyerhof *et al.*, 1992), rSSTR4 (Bruno *et al.*, 1992) and rSSTR5 (O'Carroll *et al.*, 1992).

In oxen: bSSTR2.*

It should be noted that, due to almost simultaneous publication, both rSSTR4 and rSSTR5 were termed rSSTR4. Until a future revision of nomenclature, we will use the term rSSTR4 for the subtype with the prior submission date (Bruno *et al.*, 1992), which has subtype characteristics identical to those of hSSTR4 (Rohrer *et al.*, 1993). The deduced amino acid sequence from genomic DNA was confirmed at the protein level for SSTR2 by partial amino acid sequencing of a purified somatostatin receptor from rat pituitary tumor-derived cells (Hulmes *et al.*, 1992). The human genes for SSTR1, 2, 3 and 4 have been structurally characterized as intronless and localized on separate chromosomes; hSSTR1, 2, 3 and 4 were assigned to chromosomes numbered 14, 17, 22 and 20, respectively (Yamada *et al.*, 1993). However, recent studies suggest an alternative mRNA splicing for rodent and human SSTR2 (Vanetti *et al.*, 1992; Patel *et al.*, 1993). The two isoforms

*Xin, W. W., Wong, M.-L., Rimland, J., Nestler, E. J. and Duman, R. S. (1993) Characterization and functional expression of a somatostatin receptor isolated from locus coeruleus. Submitted for publication.

†Yasuda, K., Espinosa, R., III, Davis, E. M., LeBeau, M. M. and Bell, G. I. (1993) Human somatostatin receptor genes: localization of SSTR5 to human chromosome 20, band p11.2. *Genomics* **17**: 785–786.

generated differ in 15 amino acids at the carboxyterminus and the spliced variant protein SSTR2B is 23 residues shorter.

The known SSTRs vary in size, with rSSTR1 ,2, 3, 4 and 5 being made up of 391, 369, 429, 384 and 383 amino acids, respectively. Hydropathy analysis of the SSTR sequences indicates that all have seven putative membrane-spanning helical domains characteristic of G-protein coupled receptors (Fig. 3). The percentage amino acid identity among the five subtypes is around 48% identity (about 70% similarity). The highest levels of identical sequences are found in the transmembrane regions. It should be noted that SSTR1 and SSTR4 clearly form a closely related subgroup of the SSTR gene family. This is also found with respect to their pharmacological properties (see Section 5.2). Structurally, the SSTR3 sequences exhibit two special features that may account for the unique pharmacological properties of SSTR3. An insertion in its third intracellular loop is not present in the other cloned SSTRs and the putative palmitoylation site found in other SSTRs at the carboxyterminus is missing. There are also differences between the five receptor subtypes in putative glycosylation and phosphorylation sites (cf. Table 3).

5.2. Pharmacology of SRIF Receptors

The pharmacological properties of the five cloned somatostatin receptor subtypes are different. Competition binding experiments using iodinated SRIF-14 as specific radioligand revealed high affinity binding of SRIF-14, as well as SRIF-28, to SSTR1–5. Surprisingly, octreotide exhibits high affinity binding to SSTR2 ($IC_{50} = 0.3$ nM), but has low or no affinity for SSTR1 and SSTR4 ($IC_{50} > 1000$ nM) and only moderate affinity for SSTR3 ($IC_{50} = 30$ nM). The recently cloned fifth subtype (O'Carroll *et al.*, 1992) has similar pharmacological characteristics to those of SSTR2, with high affinity binding of octreotide ($IC_{50} = 0.2$ nM).

The binding profiles of other short synthetic SRIF analogs (RC-160, BIM 23014 and MK678) are nearly identical to that of octreotide. The binding data suggest that octreotide and similar cyclooctapeptides are selective ligands for SSTR2 and SSTR5.

The somatostatin receptors have been shown to be coupled to different effector systems of signal transduction. A classical action of SRIF is the inhibition of adenylyl cyclase activity. This

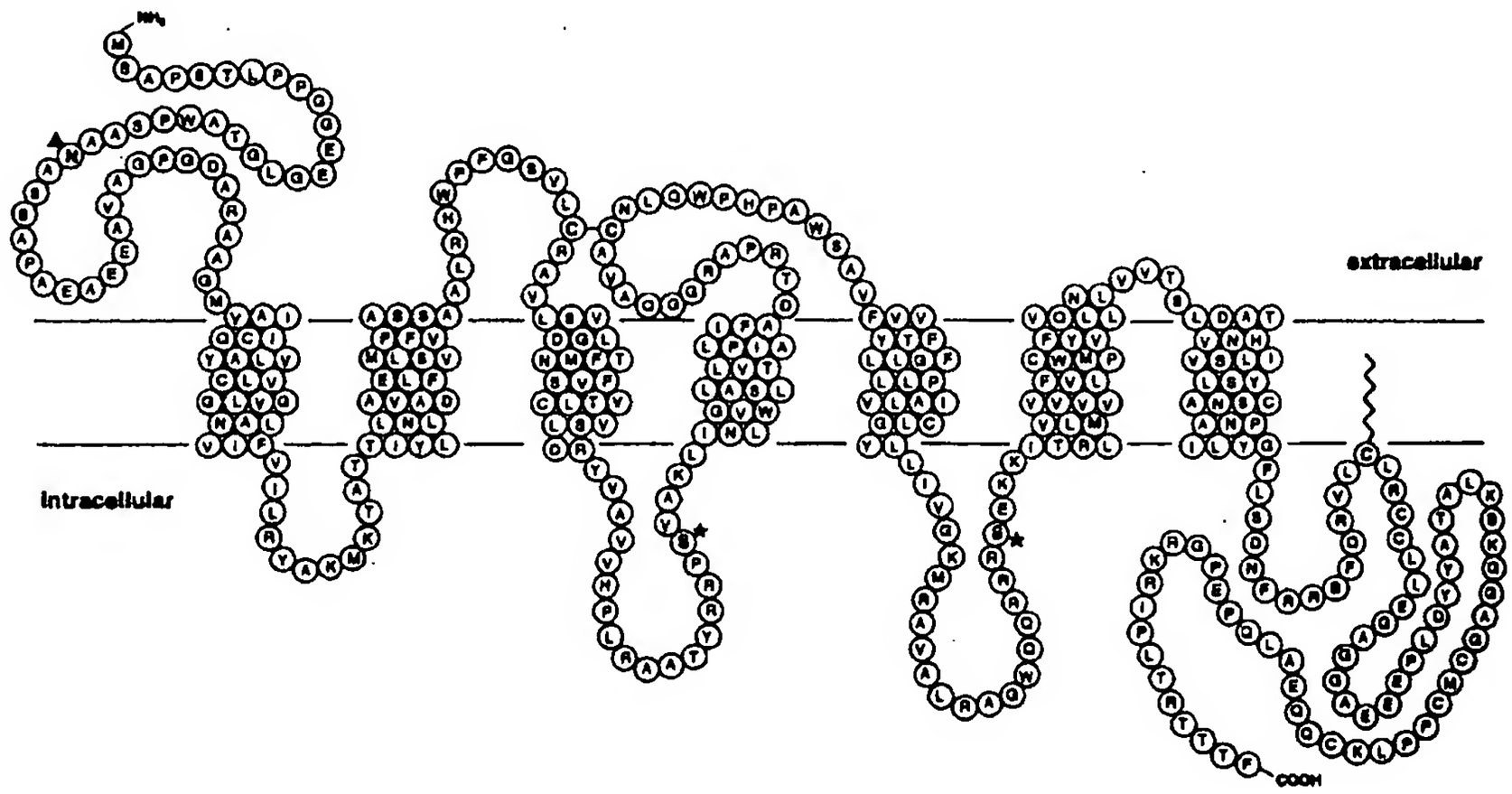

Fig. 3. Schematic model of the seven transmembrane structures of hSSTR4 (Rohrer *et al.*, 1993). The putative N-linked glycosylation site at position Asn-24 is marked. The potential phosphorylation sites at Ser-161 and Ser-253 in the second and third cytoplasmic loops are indicated by stars.

TABLE 3. *Properties of the Five Subtypes of Somatostatin Receptors*

	SSTR1	SSTR2	SSTR3	SSTR4	SSTR5
Cloned genes	human mouse rat	human mouse rat	human mouse rat	human — rat	— — rat
Chromosomal localization human	14	17	22	20	nd
Splice variants	—	SSTR2A + B	—	—	—
Putataive glycosylation sites of hSSTRs	3	4	2	1	2 (rat)
Potential phosphorylation sites of hSSTRs	2	1	6	2	8 (rat)
Putative palmitoylation	yes	yes	no	yes	yes
Tissue distribution human	brain, lung GI tract	brain, kidney pituitary, GI tract	brain,	brain, pancreas	pituitary lung
Effector system	adenylyl cyclase	adenylyl cyclase, K^+/Ca^{2+} channels	adenylyl cyclase	adenylyl cyclase	adenylyl cyclase

nd, not determined.

was demonstrated, recently, when SSTR3, 4 and 5 were transiently or stably transfected in various cell lines (Yasuda *et al.*, 1992; Kaupmann *et al.*, 1993; O'Carroll *et al.*, 1992). In contrast to previous reports (Bell and Reisine, 1993), we and others (Strnad *et al.*, 1993; Kaupmann *et al.*, 1993) also were able to demonstrate for SSTR1 and SSTR2 that SRIF inhibits dose-dependently the forskolin-stimulated increase in cAMP levels, suggesting that SSTR1–5 are coupled to the adenylyl cyclase system. The inhibitory effect of somatostatin was abolished by preincubation with pertussis toxin, indicating a coupling via a pertussis toxin sensitive G-protein. An inhibitory coupling of SSTR2 to voltage dependent Ca^{2+} channels (Scherübl *et al.*, 1992) and Ca^{2+}- and voltage-activated K^+ channels (Yatani *et al.*, 1987; White *et al.*, 1991) was also shown, which suggests a possible coupling of SSTR2 to distinct cellular effector systems via different G-proteins in different cell types (Bell and Reisine, 1993).

Molecular cloning has revealed the presence of five structurally related somatostatin receptors. In the context of this review, the question arises: what is the relevance of these SRIF receptor subtypes for the antiproliferative action of SRIF analogs? To study this question, the SRIF receptor subtype SSTR2, which binds octreotide, BIM-23014 and RC-160 with high affinity, was stably expressed in NIH3T3 cells (Buscail *et al.*, 1993). Octreotide bound with high affinity to the transfected cells and inhibited their proliferation by up to 66%. In contrast, untransfected cells were not affected by octreotide. These data are highly important since SSTR2 is apparently the predominant subtype expressed in SRIF receptor-positive tumors. Accordingly, we have recently detected, by means of RT-polymerase chain reaction, Northern blotting and receptor-binding studies, high levels of SSTR2 in rat pancreatic tumor cells (AR42J), which are potently growth-inhibited by SRIF analogs (Fig. 2). Further studies are required to clarify the role of SSTR3 and SSTR5, which may also mediate inhibitory effects of octreotide, BIM 23014 and RC 160 on tumor growth. SSTR3 and SSTR5 bind these analogs with intermediate and high affinity, respectively.

6. CONCLUDING REMARKS

Somatostatin (SRIF) is widely distributed throughout the central nervous system and the gastroenteropancreatic system. SRIF is an important regulator of neural activity, as well as being a modulator of endocrine and exocrine secretion. In addition, SRIF and SRIF analogs effectively inhibit the proliferation of various types of tumor cells. All these actions are mediated by specific, high affinity membrane receptors on target tissues, such as brain, pituitary, pancreas, gastrointestinal tract or tumors.

SRIF appears to be an important hormonal regulator of tumor cell growth. *In vitro* and *in vivo* studies have demonstrated an antineoplastic activity of SRIF and SRIF analogs. This activity can be attributed either to a direct antiproliferative effect on SRIF receptor-positive tumors or an indirect effect, whereby SRIF and SRIF analogs lower GH and IGF-1, as well as gastrointestinal hormones and enzymes. SRIF receptors were shown to be expressed on a variety of human tumors, including endocrine pancreatic tumors, pituitary and brain tumors, small cell lung and mammary carcinomas. Ongoing clinical trials are evaluating the antiproliferative potential of octreotide and other SRIF analogs in cancer therapy.

The expression of high affinity SRIF receptors in such a variety of human tumors might indicate a general control mechanism through which tumor cells are inhibited in their growth. However, the role of SRIF analogs in cancer therapy has yet to be established at the clinical level. SRIF analogs may prove to be useful in combination with other well-established drugs in cancer therapy.

High affinity SRIF receptors can serve as tumor markers and have led to the development of radiolabeled SRIF analogs for tumor imaging in patients. This new technique has important clinical implications for tumor staging and predicting therapeutic response to octreotide. Since the detection of tumor associated SRIF receptors could be predictive for a good response to octreotide therapy, a positive scan will be useful to consider octreotide therapy.

In addition, it may be possible to bring a therapeutically effective radiation dose to SRIF receptor-positive tumors by using β-emitter labeled SRIF analogs. However, it is necessary to carefully evaluate the beneficial effects of SRIF analog-mediated radiotherapy on tumor growth in comparison to the possible side effects due to radiation effects on normal tissue.

Early studies on binding properties of SRIF receptors suggested the existence of SRIF receptor subtypes. Recently, molecular cloning has revealed the presence of at least five SRIF receptor subtypes, SSTR1–5. The five structurally related SRIF receptors have distinct pharmacological properties. Short synthetic SRIF analogs bind with high affinity only to SSTR2 and SSTR5. The availability of the cloned SRIF receptors will allow for detailed structure–function analysis of SRIF receptors and will facilitate the development of subtype selective agonists or antagonists. Ligands specific for each receptor subtype will help to assess the physiological role of SSTR1–5. These subtype selective analogs could be useful for specific treatment of endocrine disorders and cancer.

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